necrosis leading to a cardiomyopathy with feature of hibernation. Early chemokine induction and interstitial fibrosis due to frequent sublethal ischemic episodes may have a role in mediating left ventricular dysfunction in ischemic cardiomyopathy.

4:15 p.m.

836-2 Serum Levels of Unbound Free Fatty Acids Reveal High Sensitivity for Early Detection of Acute Myocardial Infarction in Patient Samples From the TIMI II Trial

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Background: Levels of unbound free fatty acids (FFAu) have been found to increase with myocardial cellular ischemia in patients undergoing balloon angioplasty (Kleinfeld et al, Am J Cardiol 1996;78:1350). To assess whether FFAu are an effective marker of ischemia in acute myocardial infarction (AMI), levels of FFAu were determined in serum samples from patients enrolled in the TIMI II trial (TIMI Study Group, N Engl J Med 1989;320:618). Methods: Patients in this trial were treated with tissue plasminogen activator (t-PA) for AMI. Blood samples were drawn upon presentation, and then 50 minutes, 5 h and 8 h after t-PA. These samples have been maintained at -70°C by the National Heart Lung Blood Institute. FFAu measurements and partial data analysis has been completed on 458 patients (75 F, 383 M). Measurements were done at 22°C using the ADIFAB2 (Richieri et al, J Biol Chem 1996;271:11291) fluorescent probe of FFAu (healthy controls = 2.6 ± 0.6 nM). Results: FFAu values for the TIMI patients ranged from 2 to 5000 nM. Average values and standard deviations for each of the 4 blood draws, from time of admission to 8 h were: 13 ± 17, 22 ± 25, 11 ± 13, and 10 ± 11 (nM). These results indicate, relative to the control population, an approximately 4 fold increase upon admission, a further 2 fold increase following t-PA with a gradual decrease within 5 h of t-PA. Using a 5 nM cutoff, the predicted sensitivity for detection of AMI was 91% using samples at time of admission only, and 98% using time of admission and the 50 minute sample. Only 19 % of patients had elevated levels of creatine kinase on admission. Specificity was estimated as 93% by comparison with a distribution that includes healthy individuals plus patients with non cardiovascular diseases. FFAu values for samples drawn at presentation were found to be highly ($p<0.025$) correlated with mortality; an increase of 4 fold in mortality rate is predicted from lowest to highest FFAu levels. Conclusions: These data indicate that levels of FFAu are: 1) a sensitive indicator of ischemia in AMI, 2) elevated well before markers of cardiac necrosis, 3) an indictor of reperfusion therapy, and 4) a predictor of mortality in these patients.

4:30 p.m.

836-3 A Cardioprotective Agent, JTV519, Inhibits Apoptotic Cell Death of Postischemic Reperfused Myocardium Through _PKC-Mediated ERK Activation

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Objective: JTV519 (JTV), a 1,4-benzothiazepine derivative, confers cardioprotective effects on the post-ischemic, reperfused myocardium through a specific activation of δ-isoform of protein kinase C (PKC) (Inagaki et al., *Circulation*, 2000). In this study, we further tested the hypothesis whether the downstream of δ-PKC activation may link to its inhibitory effects on the apoptotic cell death. **Methods:** Hearts were isolated from male SD rats (n=7, for each group), and were coronary-perfused in a Langendorff setup at a constant coronary flow under electrical pacing at 3.33Hz. The preparation was treated with 1μM JTV or vehicle for 5 min, and then subjected to a 30-min global ischemia and the subsequent 60-min full reperfusion protocol. On the other hand, isolated ventricular myocytes from neonatal rats (n=6, for each group) were treated with JTV or vehicle before an exposure to 50μM H_2O_2 for 80 min. Both the heart and cell preparations were subjected to TUNEL staining, DNA fragmentation assay, and immunoblotting for phosphorylated forms of JNK, ERK1/2, and p38-MAPK. **Results:** In the reperfused heart preparations, JTV ameliorated the recovery of left ventricular developed pressure by 80% ($p<0.01$), which was associated with a reduction of TUNEL-positive ventricular myocytes by 10% ($p<0.05$). In isolated cells, JTV reduced the H_2O_2-induced TUNEL staining from 43% to 30% ($p<0.05$). JTV also showed substantial decreases of DNA fragmentation in both preparations. These anti-apoptotic effects of JTV were inhibited either by GF109203X (GF, a PKC inhibitor) or by PD98059 (PD, a MEK inhibitor). In the cell preparations, JTV further increased H2O2-induced phosphorylation of ERKs by 55% ($p<0.01$). This ERK activation was decreased by pretreating the system with 50μM PD, 5μM GF, and 1μM rottlerin(a δ-PKC specific inhibitor) to the levels of 18.0%, 55.2%, and 31.5%, respectively ($p<0.05$). JTV did not show significant effects on JNK and p38-MAPK phosphorylation. **Conclusion:** JTV519 protects the post-ischemic reperfused myocytes from the apoptotic cell death through a specific activation of the δ-PKC/ERK cascade.

4:45 p.m.

836-4 Hearts of Stat 6 Knockout Mice Are Resistant to Ischemia Reperfusion Injury

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Background: STATs (Signal transducers and Activators of Transcription) comprise a family of transcription factors that reside in the cytoplasm of resting cells. Recently, ischemia/reperfusion was found to rapidly activate JAK (a group of tyrosine kinases)/STAT signaling pathway which play a crucial role in myocardial ischemic injury. Specifically, JAK2 and STAT 6 were activated even after 15 min ischemia and remained activated during subsequent reperfusion.

Methods: To confirm the role of STAT 6 in ischemic injury, we examined if STAT 6 knockout mice devoid of any copies of STAT 6 gene was resistant to ischemic injury. STAT 6 knockout mice (n=10) and control wild-type mice (n=10) were anesthetized with pentobarbital, hearts excized, and perfused via working mode with KHB buffer. The working mouse hearts were made globally ischemic for 25 min followed by 2 h of reperfusion. Left ventricular function was monitored at the baseline and during post-ventricular reperfusion and infarct size was determined at the end of the reperfusion.

Results: Heart rates of the STAT 6 knockout (KO) mice were lower compared to wild-type (WT) mice at baseline and at 15 min of reperfusion (15R). Knockout mouse hearts displayed significantly better ($p<0.05$) post-ischemic ventricular recovery as evidence by higher left ventricular pressure (LVP) (mm Hg) [BL: 86.5±0.7 (WT) vs. 97.3±1.7 (KO); 15R: 75.2±1.4 (WT) vs. 82.2±2 (KO); 30R: 71.8±1.4 (WT) vs. 77.8±2.1 (KO)]; higher dp/dtmax (mm Hg/sec) [BL: 4518±76 (WT) vs. 5085±120; 15R:3807±130 (WT) vs. 4288±134 (KO); 30R: 3529±131 (WT) vs. 4026±133 (KO). Infarct size determined by TTC staining was lower in STAT 6 KO mouse hearts (41.1±1.6%) compared to those for the WT mice (48.6±2.3%).

Conclusion: The results of this study demonstrate that hearts of STAT 6 knockout mice are resistant to ischemia/reperfusion injury suggesting a role of STAT 6 gene in the mediation of cardiac injury.

5:00 p.m.

836-5 Glucagon-Like Peptide-1 (GLP-1) Limits Myocardial Stunning Following Acute Coronary Occlusion and Reperfusion in Conscious Canines

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Background: Myocardial ischemia is associated with increased glucose utilization. Glucose Insulin K+ infusion has been shown to improve outcomes. We hypothesized that the insulinotropic peptide GLP-1 facilitates recovery of post-ischemic contractile dysfunction (stunning).

Methods: We studied 13 conscious dogs, instrumented with LV pressure gauges, aortic and coronary flow (CBF) probes, piezoelectric crystals to measure regional systolic wall thickening (WTh) and a hydraulic coronary artery occluder. All dogs underwent a 10 min coronary occlusion (CAO) followed by reperfusion (CAR). Five dogs received a 24-hr infusion of GLP-1 (1.5 $pmol \cdot kg^{-1} \cdot min^{-1}$) initiated 1 min prior to CAR; 8 dogs received placebo. We obtained serial recordings for the first 3 hrs and at 24-hr post-CAR.

Results: Hemodynamic responses and global LV function during CAO and CAR were similar in the two groups. Baseline regional WTh (2.6±0.3mm) and initial hyperemic responses were similar. Despite comparable changes in CBF, GLP-1 treated dogs demonstrated significantly (*$p<0.01$) less post-ischemic contractile dysfunction. The salutary effects of GLP-1 were sustained for 24-hrs.

	Regional CBF (ml)		Regional WTh (% baseline)	
	Control	GLP-1	Control	GLP-1
Baseline	21±1	20±2	100%	100%
CAO	3±2	5±2	12±8%	16±11%
CAR-1min	113±9	93±11	76±6%	84±8%
5 min	49±8	41±9	60±4%	81±4%*
10 min	22±2	18±2	57±5%	84±2%*
15 min	18±1	17±2	57±5%	87±2%*
30 min	19±2	19±2	60±6%	86±3%*
1 hr	17±1	18±3	69±5%	97±3%*
2 hr	17±2	18±2	70±5%	97±3%*
3 hr	17±2	17±3	76±6%	98±2%*
24 hr	22±2	22±1	78±4%	98±4%*

Conclusions: When administered at the time of reperfusion, GLP-1 limits myocardial stunning following brief coronary occlusion. The salutary outcome is sustained for at least 24 hrs. GLP-1 may be a promising adjuvant therapy in post-ischemic myocardial dysfunction.

5:15 p.m.

836-6 Cardiac Serum Marker Release After Percutaneous Transluminal Septal Myocardial Ablation: A Human Model

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Background. Multiple cardiac serum markers are available for determining myocardial cell death, but the comparative kinetics of these markers in humans have not yet been fully described due to the inability to precisely correlate symptom onset with vessel closure. Percutaneous transluminal septal myocardial ablation (PTMSA) for hypertrophic obstructive cardiomyopathy permits a unique opportunity to identify the time of vessel closure in a series of patients experiencing a MI and compare the course of cardiac serum marker elevation.

Methods. We obtained blood samples every 2 hrs for 48 hrs from 11 pts after PTMSA and measured the serum markers: creatine kinase MB (CK MB)(normal 0.0-8.8 ng/ml), myoglobin (30-90 mg/L), troponin-I (0.0-2.0 ng/ml), and troponin-T (0.0-0.1 ng/ml).

Results. Mean marker values are displayed in the figure below. In all patients studied, all markers were elevated above normal by 2 hrs after vessel closure. Mean peak measure-